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Clinical Observations, Virologic Studies, and Treatment Trials in Patients wih Epidermodysplasia Verruciformis, a Disease Induced by Specific Human Papillomaviruses

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We have studied 11 patients with the papillomavirus-induced disease epidermodysplasia verruciformis (EV). Clinical diagnostic features are widespread, long-lasting, pityriasis versicolor-like macules and flat, wart-like papules, both usually occurring in early childhood, with the subsequent development in the third decade of multiple skin cancers of the Bowenoid in situ and squamous cell types, primarily in sun-exposed skin. Virologic studies using the methods of immunofluorescence microscopy, restriction endonuclease analysis, and DNA blot hybridization have shown benign lesions to be associated with one or several types of the human papillomaviruses (HPVs) specifically associated with EV (at least 15 types recognized on the basis of sequence homology studies of molecularly cloned genomes). Skin cancers in these patients were associated with the genomes of either HPV-5, HPV-8 or HPV-14, suggesting that these three viruses are potentially oncogenic. A genetic factor appears to play a role in the pathogenesis of EV, since 5 of our patients were children of consanguineous parents and 2 had siblings also suffering with EV, suggesting a recessive inheritance pattern. Treatment of 4 EV patients with an oral retinoid resulted in partial temporary improvement of benign lesions, and the treatment of 2 patients with intralesional interferon injections into multiple Bowenoid cancers in situ has resulted in the disappearance of these lesions. Finally, EV serves as a model for studying the interplay of oncogenic viruses, genetic and immunologic factors, and sunlight in the production of skin cancer in humans.

Epidermodysplasia verruciformis is one of the human diseases in which there is considerable evidence for an etiologic role of oncogenic papillomaviruses [1]. Genital cancers have also recently been shown to be associated with papillomaviruses ([2] and L. Grissman et al, This Symposium). Other probably oncogenic virus-induced diseases are EBV-associated Burkitt's lymphoma and nasopharyngeal carcinoma, hepatitis B virus-associated hepatocellular carcinoma, and HTLV-associated T-cell lympholeukemias [3]. EV of Lewandowsky and Lutz [4] is characterized by the appearance in childhood of refractory, widespread pityriasis (tinea) versicolor-like (PV-like) and flat, wart-like (FW-like) skin lesions [5] induced by human papillomavirus types 3, 5, 8, 9, 10, 12, 14, 15, 17, 19–25 ([1,6–11] and G Orth et al, This Symposium); the appearance usually in the third decade of multiple Bowenoid cancers in situ and squamous cell invasive cancers occurring primarily in sun-exposed skin; parental consanguinity and sibling involvement in some patients [5]; mental retardation in some [5]; and depressed cell-mediated immunity (CMI) in most [12]. We have studied 11 EV patients and will report here our clinical observations, genetic, virologic and immunologic studies, and experience with treatment trials using an oral aromatic synthetic retinoid and interferon injections.

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Abbreviations:
CMI: cell-mediated immunity
EBV: Epstein-Barr virus
EV: epidermodysplasia verruciformis
FW-like: flat wart-like
HTLV: human T-cell leukemia virus
HPV: human papillomavirus
MEM: minimum essential medium (Eagle's)
PV-like: pityriasis versicolor-like

PATIENTS STUDIED

Five of the 11 patients in this series were referred to us for study from dermatology clinics throughout France. One of these was born in Guadeloupé and another in the Congo. Three were referred to us from Algeria, two from Italy, and one from Lebanon (Tables I and II).

METHODS

Specimen Collection

Scales of benign lesions were scraped with a curette, and the scrapings were frozen in liquid nitrogen and stored at −70°C. Repeated scrapings were taken from some patients weekly. Those which could not be frozen immediately were transported in Eagle's minimum essential medium (MEM) containing antibiotics and later frozen. Biopsies of benign lesions and skin cancers were either processed immediately or transported in Eagle's MEM and later processed.

Light and Electron Microscopy

Biopsies were divided into portions. The portion for light microscopy was fixed in Bouin's fluid and embedded in paraffin, and sections were stained with hematoxylin and eosin (H&E). The portion for electron microscopy was fixed in buffered 4% glutaraldehyde, postfixed in 1% osmium tetroxide, dehydrated in upgraded alcohols, and embedded in an Epon-Araldite mixture. One-micron sections for light microscopic observation were stained with methylene blue-basic fuchsin; thin sections for electron microscopy were stained with uranyl acetate and lead citrate.

Immunofluorescence Microscopy

The portions for immunofluorescence studies were frozen in liquid nitrogen, and cryostat sections were cut and fixed in acetone. These sections were treated by the direct method by incubating with fluorescein-labeled anti-HPV-1 or HPV-2 guinea pig IgG or by the indirect method by using an anti-HPV-3 guinea pig antiserum or an antiserum produced by injecting viral particles purified from pooled scrapings from an EV patient infected with 10 specific EV HPVs (G Orth et al, in preparation), followed by fluorescein-labeled anti-guinea pig IgG rabbit IgG as described previously in detail [13].

Preparation of Viral and Cellular DNA

Viral DNA was extracted from benign lesions by a modified Hirt's method [1,14], and total DNA was extracted from cancers by a method previously described [1].

Restriction Endonuclease Analysis

Digestion of DNA preparations by *Pst*I, *Bam*HI, or *Hin*dII endonucleases (Boehringer, Mannheim) and the separation of cleavage products by electrophoresis in agarose (1.2%) vertical slab gels were per-

TABLE I. *Clinical observations*

Patient	Age when first seen (years)	Sex	Age of onset of benign lesions (years)	Age of onset of cancer (years)	Time from wart to cancer (years)	Cancers observed		
						Type	Number	Location
1	40	M	6	25	19	Squamous cell	1	Forehead (invading orbit and sinuses)
						Squamous cell	1	Preauricular
2	32	M	2	30	28	Bowenoid in situ	15	Scrotum
						Squamous cell	4	Palm
						Squamous cell	4	Buttocks
						Squamous cell	1	Scrotum
3	52	F	10	47	37	Bowenoid in situ	2	Forehead
						Squamous cell	2	Forehead, temple
4	31	M	5	30	25	Bowenoid in situ	2	Forehead
						Bowenoid in situ	1	Preauricular
						Squamous cell	1	Forehead
5	28	M	6	—		None		
6	40	M	6	36	30	Bowenoid in situ	1	Forehead
7	33	M	1	24	23	None		
8	38	F	?	—	—	None		
9	32	M	10	26	16	Bowenoid in situ	1	Forehead
						Squamous cell	1	Perianal, anal canal
10	21	M	?	—	—	None		
11	50	M	5	40	35	Bowenoid in situ	3	Forehead

TABLE II. *Genetic observations*

Patient	Nationality	Parental consanguinity	Siblings affected	Total in sibship	Other information
1	French	No	1	2	
2	Congolese	First cousins	3	5	One sister with PV-like lesions and no cancers; one brother with FW-like lesions and no cancers.
3	French	No	2	3	One brother with PV-like lesions; multiple skin cancers, treated by x-rays; died; cause?
4	Algerian	Cousins	?	?	Married to a cousin; children normal.
5	Algerian	First cousins	1	9	Three siblings died in childhood; cause?
6	Algerian	No	1	2	
7	Italian	No	1	2	
8	Italian	Second cousins	1	3	
9	Lebanese	Cousins	1	6	Grandparents were also cousins.
10	French[a]	No	1	5	
11	French	No	1	4	

[a] Born in Guadeloupe.

formed as previously described [1,10,11]. Molecular weights of fragments were evaluated by comparing their electrophoretic mobilities in ethidium bromide-stained gels with mobilities of *Hin*dIII λ phage DNA fragments [1,10].

DNA Blot Hybridization

DNA fragments were depurinated and denatured in situ [1,10,11] and then transferred on Gene Screen membranes (New England Nuclear Corp., Boston, Mass.). ^{32}P-labeled DNA probes prepared from cloned EV HPV DNAs (HPV types 3, 5, 8, 9, 10, 12, 14, 15, 17, 19–24) ([10,11] and G Orth et al, This Symposium, and D Kremsdorf et al, in preparation) were labeled by nick translation according to a previously described method [1,10]. Blots of DNA obtained from benign lesions and cancers were incubated with all the DNA probes under stringent hybridization conditions as previously described [11]. Hybrids were detected by autoradiography [10].

ANALYSIS OF CLINICAL OBSERVATIONS

Benign Lesions

Benign lesions had an average age of onset of 6 years (Table I). The most common benign lesions in our EV series were small (2–8 mm), slightly scaly macules that were various shades of brown, red, or white and which sometimes coalesced into large patches with polycyclic borders. The brown and white lesions resembled the common dermatophyte-induced disease pityriasis versicolor in both appearance of the macules and patches and their distribution on the trunk, shoulders, neck, arms, and face, thus the designation pityriasis versicolor-like (PV-like) (Fig 1). The red lesions have been called "scaly red plaques" in previously reported descriptions [13]. PV-like lesions were found in all patients in our series. Another common lesion occurring in all but one of our patients was the slightly scaly papule, small (2–6 mm), flesh-colored, pink, red, tan, brown, or white, and sometimes black in dark-skinned patients. Papules sometimes coalesced into plaques. These papular lesions were most often seen on the backs of the hands, on the wrist and forearms, and occasionally on the trunk or face, and they resembled flat warts in appearance, therefore the designation flat wart-like (FW-like) lesions (Fig 2). Less commonly seen were psoriasiform plaques on the elbows and knees and porokeratosis-like lesions on the limbs. One patient, in addition, had common warts and typical flat warts on the hands and forearms.

Cancers

Skin cancers were found in 7 of our 11 EV patients (Table I). The stage of cancer most commonly seen was the Bowenoid cancer in situ found in 6 patients. These appeared most commonly as red, brown, or black plaques, often located on the face (Fig 3), especially the forehead, and rarely on non-sun-exposed skin. An exception to this was our black African patient who had multiple lesions on his scrotum. The other stage of cancer seen was the more advanced squamous cell carcinoma present in 5 patients. These lesions also occurred most commonly on sun-exposed skin, especially the forehead (Fig 3), again with

the exception of the Congolese patient with cancers on his palms (Fig 4) and buttocks. One of his palmar cancers was observed to develop over an 18-month period from a dark brown pigmented macule (Fig 5). Our Lebanese patient had a perianal cancer. Patient 4 recently developed a metastasis in a cervical lymph node.

HISTOLOGY OF LESIONS

Light Microscopy

A histologic pattern characteristic for EV was seen in biopsies of PV-like and FW-like benign lesions from all patients (Fig 6). Characteristic cells in the stratum spinosum and stratum granulosum were enlarged and had pale-staining cytoplasms containing only round keratohyalin granules. These cells had clear spaces in their nuclei [15]. Parakeratotic cells were common in the stratum corneum. In contrast to macular lesions, papular lesions showed moderate acanthosis.

Bowenoid cancer in situ lesions were acanthotic. Epidermal cells had lost their polarity, were dyskeratotic, and had hyperchromatic nuclei and abnormal mitotic figures. No dermal invasion was seen (Fig 7). Squamous cell cancers were characterized by invasion of the dermis by single epidermal cells or nests of epidermal cells (Fig 8), sometimes with Bowenoid features, i.e., dyskeratotic cells with hyperchromatic nuclei and abnormal mitotic figures.

Electron Microscopy

PV-like and FW-like lesions were examined from 2 patients. Ultrastructurally, the characteristic pale-staining cells had round keratohyalin granules but few tonofilament bundles in

FIG 2. Papules covered with a whitish scale on the back of the hand of patient 1. These flat papules are examples of flat wart-like (FW-like) lesions typical for EV.

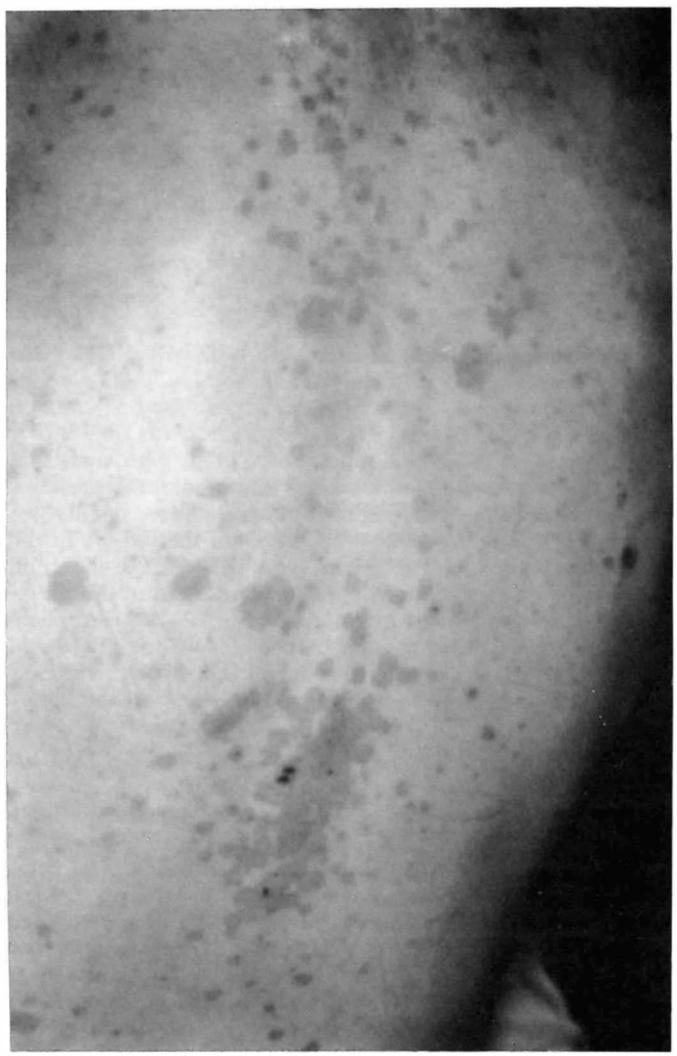

FIG 1. *A*, Slightly scaly macules on the chest of patient 1. These are pityriasis (tinea) versicolor-like (PV-like) lesions, typical of benign EV lesions. *B*, Depigmented PV-like macules on the back of patient 2. Some of the lesions are coalescent and have polycyclic borders.

FIG 3. Face, neck, and upper back of patient 4 showing a large ulcerated squamous cell carcinoma of the forehead, brownish plaques on the forehead, temple, and preauricular regions that are Bowenoid cancers in situ, and white and pink macules on the back and neck that are PV-like lesions.

FIG 4. The palm of patient 2 showing a large invading squamous cell cancer of the thenar eminence. Another smaller squamous cell carcinoma is seen at the base of the fourth finger, and brown scaly benign macules are seen on the palm and fingers.

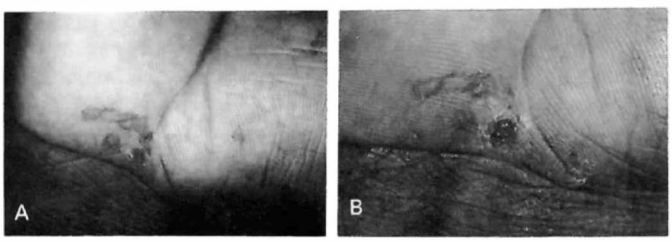

FIG 5. *A*, The other palm of patient 2 showing a cluster of five brown macules. *B*, The same cluster of lesions 18 months later. A fungating squamous cell carcinoma has developed at the site of one of the macules.

the almost empty cytoplasm (Fig 9). Nuclei of these cells contained papillomavirus-like particles arranged in paracrystalline arrays. Nuclear clear spaces contained fine filaments, also seen to be present in the cytoplasm. No virus particles were found in the one Bowenoid cancer in situ and the one squamous cell cancer examined.

GENETIC OBSERVATIONS

In our series, 45% of the patients were children of a consanguineous marriage (Table II), two first cousin marriages, one second cousin marriage, and the other two of unknown degree. Two of the consanguineous marriages were in families from small Algerian villages, where consanguinity is not uncommon. In fact, one of the three Algerian patients was married to his cousin; his children are still young and to date have no evidence

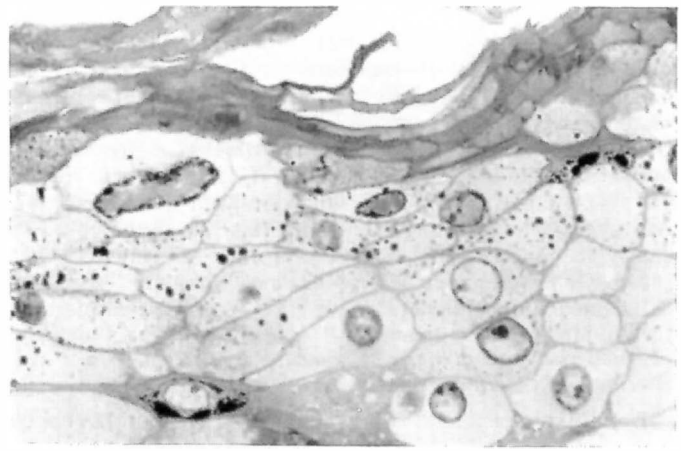

FIG 6. Histologic section of a PV-like lesion of patient 2. Cells showing the characteristic features of benign EV lesions are seen. These are enlarged cells with pale-staining cytoplasm containing rounded keratohyaline granules. Nuclei show clear spaces, and some nuclei have inclusions (plastic-embedded, methylene blue-basic fuchsin stained 1-μm-thick section).

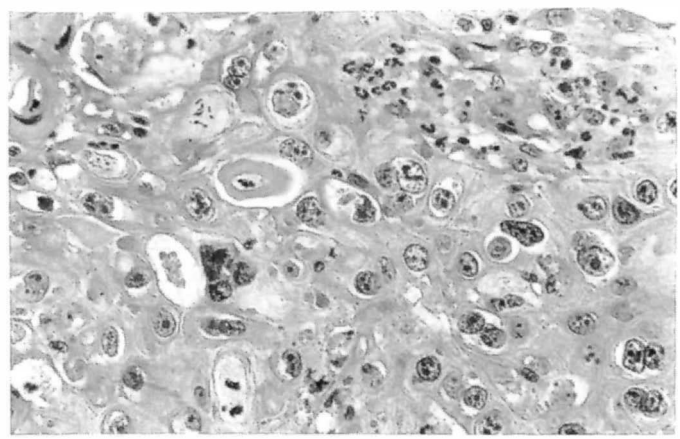

FIG 7. Histologic section of a Bowenoid cancer in situ from an EV patient. Note the loss of a cellular polarity, dyskeratosis, and abnormal nuclei that are hyperchromatic and bi- or multinucleated (paraffin-embedded, H&E stained).

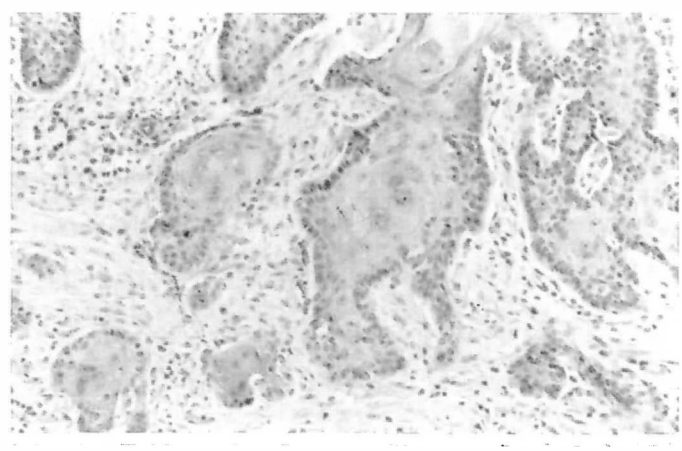

FIG 8. Histologic section of an invasive squamous cell carcinoma from an EV patient. Islands of epithelial cells are seen in the dermis. Basal cells form the periphery and the interior cells are differentiating (paraffin-embedded, H&E stained).

of EV. One other consanguineous family was from a village in the Congo. This family had 3 of 5 children involved with EV. The other consanguineous families were Lebanese and Italian.

VIROLOGIC STUDIES

Immunofluorescence Microscopy

Biopsies of benign lesions were studied by immunofluorescence microscopy. Biopsies taken from PV-like and/or FW-like lesions of all 11 patients were immunofluorescence-positive for an antiserum prepared against a mixture of 10 EV HPVs found in scrapings from a single patient (G. Orth et al, in preparation), indicating the presence of structural viral proteins of EV HPV(s). Biopsy of a typical flat wart from one patient was immunofluorescence-negative using this antiserum, but was shown to contain HPV-3 structural antigens using an antiserum prepared against HPV-3 virions. A common wart from this same patient was positive for HPV-2 antigen, but negative for EV HPVs.

Biochemical Studies

Detailed characterization of the HPV DNAs found in benign lesions and cancers of these EV patients will be reported elsewhere (D Kremsdorf et al, in preparation, and G Orth et al, in preparation).

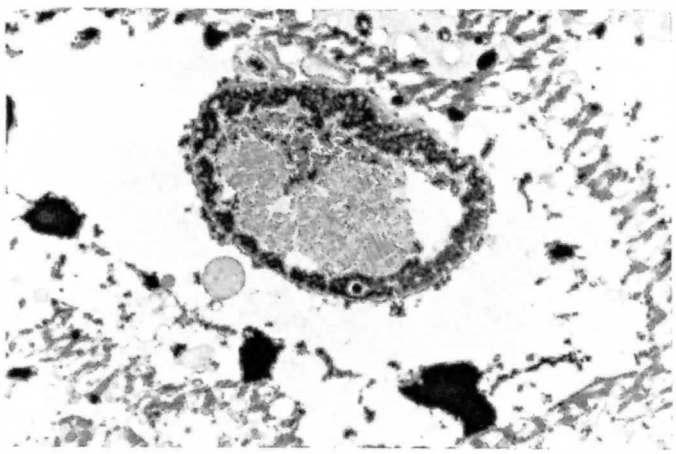

FIG 9. Electron micrograph from PV-like lesion of patient 2 showing a cell of the granular layer. The nucleus contains papillomavirus particles in a crystalline array and an adjacent nuclear clear space. The cytoplasm is nearly empty except for a few keratohyalin granules.

Benign Lesions

Fragments generated after cleavage of the DNA extracted from the lesions by *Pst*1, *Bam*HI, and *Hin*dII endonucleases were separated by gel electrophoresis and observed after ethidium bromide staining and/or after blot hybridization experiments performed under stringent conditions using probes specific for prototypical EV HPVs. HPV-5 [10] was detected in the DNA extracted from the lesions of 5 patients, HPV-8 [11] in 3 patients, HPV-20 in 2 patients, and HPV-14 and HPV-22 each in 1 patient. These five viruses show a cross-hybridization ranging from 6 to 30% (D Kremsdorf et al, in preparation, and G Orth et al, in preparation). DNAs cross-hybridizing partially with HPV-5 or HPV-9 probes and showing cleavage patterns distinct from known HPVs were found in 1 and 3 patients, respectively. Finally, HPV DNAs with cleavage patterns distinct from known HPVs and showing no cross-hybridization with known probes were found in 2 patients. Four patients were found infected with one HPV, 4 patients with two HPVs, 1 patient with three HPVs, and 1 patient with five HPVs (Table III).

Cancers

Blot hybridization experiments using these same EV HPV probes were performed with DNA extracted from three Bowenoid cancers in situ and four squamous cell cancers removed from 4 of the patients (Table III). Genome copies of HPV-5 were found in two squamous cell cancers of patient 4 (Fig 3) and in a Bowenoid cancer in situ from patients 3 and 6. Genome copies of HPV-8 were identified in both a squamous cell cancer of the palm (Fig 4) and a Bowenoid cancer in situ from the scrotum of patient 2. Genome copies of HPV-14 were found in the forehead squamous cell carcinoma of patient 1 (Table III).

IMMUNOLOGIC OBSERVATIONS

None of 3 patients tested could be sensitized to DNCB (patients 1–3). Helper/suppressor T-lymphocyte ratio was normal in the 2 patients tested (patients 2 and 3) (this test was performed by L LaRoche). The number of T- and B-lymphocytes as measured by E and EAC-rosetting and their in vitro response to antigens and nonspecific mitogens were normal in patients tested (patients 3 and 4). Blood immunoglobulin levels were normal in the patients in whom this was measured (patients 1–4).

TABLE III. *HPV types identified in benign lesions and cancers of EV patients*[a]

Patient[b]	Scrapings of benign lesions		Biopsies of cancers		
	Minimum number of HPVs	HPV type	Cancer type	Location	HPV type
1	1	14	Squamous cell cancer	Forehead	14
2	1	8	Bowenoid cancer in situ	Scrotum	8
			Squamous cell cancer	Base of thumb	8
3	2	5, 22	Bowenoid cancer in situ	Forehead	5
4	1	5	Squamous cell cancer	Forehead	5
			Squamous cell cancer	Preauricular	5
5	5	3, 5, 20, 2 PVs not as yet classified[c]			
6	1	5	Bowenoid cancer in situ	Forehead	5
7	3	5, 20, PV not as yet classified			
9	2	5-related,[d] 9-related			
10	2	8, 9-related			
11	2	8, 9-related			

[a] As determined by DNA blot hybridization experiments.

[b] No scrapings available from patient 8.

[c] Not as yet classified means an HPV DNA yielding distinct restriction enzyme cleavage patterns on agarose gels and showing no cross-hybridization with known probes.

[d] HPV 5- and 9-related refer to those viruses not as yet classified, which cross-hybridize with HPV-5 or HPV-9 probes, respectively, to an extent less than 50%, showing that these viruses are related to but subtypes of HPV-5 and HPV-9 (G Orth et al, This Symposium).

TREATMENT TRIALS

Retinoids

Four patients were treated orally with a synthetic aromatic retinoid, etretinate (Tigason), at a dose of 1 mg/kg of body weight per day (patients 1–4). Within 3 weeks, benign macular lesions in all four became less scaly, papular lesions flattened, red plaques became less red, and the achromic lesions of patient 2 were repigmenting. However, even after continued usage (4 or more months), complete clearing was never seen, and after cessation of treatment, lesions returned to their previous abnormal state. Two small Bowenoid cancers in situ in patient 2 reduced in size but never completely disappeared. Biopsies of benign lesions taken before and after treatment of patients 1 and 2 were examined by electron and immunofluorescence microscopy and DNA extracted from pooled scrapings was analyzed by gel electrophoresis for virus quantification. In patient 2 infected with HPV-8, viral content diminished remarkably, but in patient 1 infected with HPV-14, there was no change in viral content.

Interferon treatment

Three patients were treated with human α interferon (specific activity 10^6 units/mg protein) provided by Institut Pasteur Production. In one patient (patient 2), 8 small (3–5 mm) Bowenoid cancers in situ of the scrotum were treated with intralesional injections of 10^5 units of interferon every other day for 15 injections. After the tenth injection, the lesions had disappeared. Eighteen months after stopping injections, the lesions had not returned but a few new ones had appeared. In another patient (patient 3), two Bowenoid cancers in situ of the forehead were treated, twice weekly for a total of 15 intralesional injections (10^5 units of interferon). After the last injection, the lesions had disappeared. No histologic evidence for tumor was found 1 week later on biopsy. Four months later no return of lesions was noted. In a third patient (patient 1), a large squamous cell cancer of the forehead that was invading the orbit and sinuses, resistant to surgical intervention and chemotherapy, and paradoxically worsening following radiotherapy was injected intralesionally with 10^5 units of interferon every other day, and 2.5×10^6 units was injected daily into the patient's buttock for possible systemic effect. Within 1.5 months the tumor volume had reduced about 50%; it then remained stationary for 2 months. Following an unavoidable interruption of interferon supply for 3 weeks, treatment was restarted but no further improvement was seen during 4 more months of therapy. The benign lesions remained unchanged. The cancer invaded further and the patient died from general debilitation and lung infection resistant to treatment.

DISCUSSION

Our observations and studies of 11 patients with EV allow us to discuss the clinical aspects, viral etiology, role of genetics, cell-mediated immunity, and ultraviolet radiation in the pathogenesis of this disease and possibilities of treatment.

The clinical features that permit early diagnosis, prior to the occurrence of skin cancer, are the characteristic widespread refractory macules and patches, usually appearing in childhood, which resemble pityriasis versicolor, and scaly papules especially on the back of the hands which resemble flat warts [1,5,13,15]. The characteristic histologic pattern of these lesions permits almost certain diagnosis of EV [1,15,16], and serologic and biochemical identification of EV HPV(s) and EV HPV DNA(s), respectively, confirms the diagnosis ([1,6–13] and G Orth et al, This Symposium). Early recognition of EV is important for the patient in terms of prognosis, genetic counseling, and early recognition and treatment of skin cancers.

Cancer incidence in EV has been reported to range from zero in a black Nigerian series [17], to 30% in a review of 147 patients [5], to 100% in a Colombian series [15]. In our series, the incidence was 64%, the average age of onset of cancer was 32 years, and the average time elapsed between the onset of benign lesions and cancer was 27 years. This long incubation period suggests a multistep process in cancer production. Cancers were the early Bowenoid in situ or the more advanced squamous cell types. Two of our patients (patients 1 and 2) had cancers that paradoxically worsened after receiving radiotherapy, suggesting that radiotherapy is contraindicated for treating EV cancers. Except for our black African and Lebanese patients, all cancers occurred exclusively on sun-exposed areas, most commonly the forehead. The one patient who died while under observation succumbed to general debilitation resulting from a forehead squamous cell carcinoma that had invaded his orbit and sinuses. Metastases to a cervical lymph node occurred in another patient. A previously reported [18] EV patient died of a Burkitt's lymphoma (JH Herndon, Jr, personal communication) and another one [19] died of primary liver cancer (PC van Voorst Vader and LHHM Driessen, personal communication). It may be of interest that both these diseases are considered to be, in part, virus-induced [3]. Perhaps EV patients are more susceptible to oncogenic viruses.

In the late 1960s, electron microscopic studies [15,19–21] showed benign lesions of EV patients to be infected with papillomavirus-like particles, indicating that EV was virus-induced. In the late 1970s and early 1980s, it was shown by serologic and biochemical techniques that specific human papillomaviruses (HPV types 3, 5, 8–10, 12, 14, 15, 17, 19–25) were involved in EV [1,6–11,13,22]. The full classification of HPVs identified to date includes HPV-1, primarily associated with deep plantar warts [23,24], HPV-2, HPV-4, and HPV-7 with common warts [16,25], HPV-3 and HPV-10 with flat warts [11,25], HPV-6, HPV-11, HPV-16, and HPV-18 with genital warts and neoplasias [2,26–28], and HPV-13 with focal epithelial hyperplasia [29].

To date most patients infected with HPV-3 or HPV-10 alone have not developed the full EV syndrome with skin cancers. Nevertheless, HPV-3 has been included as an EV virus, since patients with generalized flat warts infected by HPV-3 alone have occurred in two EV families [1] and since an HPV-3-related genome has been reported in one EV cancer [7]. The more classic severe form of EV accompanied commonly by skin cancer is characterized by infection with at least 14 specific HPV types. All patients in our series were found to be infected with one or more HPVs known to be associated with the severe form of EV. One patient was infected with HPV-5 alone, one with HPV-8 alone, one with HPV-14 alone, and the other eight were infected with 2 to 5 HPVs. Although HPV-5 seems to be the most common virus of the EV group, most patients are infected with multiple viruses ([1,8,10,11,13,22,30] and G Orth et al, in preparation).

The search for viral genomes in EV cancers has resulted in the identification of HPV-5 genome copies not only in primary skin cancers [1,8,30], but also in one metastatic subcutaneous cancer [8]. Viral structural proteins cannot be detected in EV cancers by serologic methods, nor can full virus particles be detected by electron microscopic studies [1,20,21]. We have studied four squamous cell cancers and three Bowenoid cancers in situ from four patients by blot hybridization using EV HPV probes and have found multiple genome copies of HPV-5 in four cancers from three patients, genome copies of HPV-8 in two cancers from one patient, and genome copies of HPV-14 in another patient. Therefore, it appears from our studies that at least three viruses of the EV group, HPV-5, HPV-8, and HPV-14, are potentially oncogenic.

That genetic factors may play a role in EV has been suggested by the finding that 10% of EV patients are the products of consanguineous marriages [5] and by the finding that more than one sibling is involved in 10% of EV families [5]. In our series we found an unusually high number of consanguineous families, 5 of 11 (45%). Two of these families were from

Algerian villages where consanguinity is common; the others were Lebanese, Congolese, and Italian. Two patients had sibling(s) with the same disease. One of these families with 3 of 5 children involved also was consanguineous. These data support the notion that most EV may result from an abnormal recessive gene, others being phenocopies resulting from environmental influences alone [5]. One such phenocopy has already been identified, an immunosuppressed renal allograft recipient with an EV-like syndrome caused by HPV-5, EV HPV viral particles being found in his benign lesions [31] and the HPV-5 genome in two of his skin cancers [32]. The abnormal product of the recessive EV gene has not yet been uncovered. Mental retardation severe enough to require institutionalization has been reported in 10% of EV patients [5]. None of our patients appeared retarded.

Cell-mediated immunity has been reported to be depressed in most EV patients [1,12], while humoral immunity remains normal. CMI was found depressed in all 4 patients in our series in which the DNCB sensitization test could be performed. Since it has been shown that chronic wart infections may produce depression of CMI [33], it is possible that the lowered CMI in EV might be secondary to chronic infection with HPV. However, it is also possible that the immunologic defect is primary, allowing increased susceptibility to EV HPVs. This is supported by the observations that patients with low CMI resulting from genetic disorders of immunodeficiency, malignancies of the lymphoid system, cancer chemotherapy, and immunosuppressive therapy, as in renal allograft recipients [34–36], all have increased susceptibility to HPVs. It has been reported that patients bearing widespread flat warts infected only with HPV-3, but no skin cancers, also have severe CMI depression [1,12]. This would suggest that although CMI might play an important role in susceptibility to virus infection, cancer development is dependent finally on whether or not the infecting virus has an oncogenic potential.

The role of ultraviolet radiation from the sun in the promotion of skin cancers in EV patients is suspected for the following reasons: (1) although benign EV lesions are widespread, skin cancers are located primarily on sun-exposed areas, especially the forehead and face [5], (2) EV patients with black skin have a low incidence of skin cancers [17], their skin pigmentation apparently affording them protection, and (3) immunosuppressed renal allograft recipients have increased incidence of warts and skin cancers, especially in sun-exposed areas, and this incidence is proportional to the degree of sun exposure [36], suggesting a synergism between ultraviolet light and immunosuppression. Our one black African patient has had multiple skin cancers, but none on sun-exposed skin, suggesting that he was protected by his pigmentation, but that some cofactor other than sunlight might be playing a role in cancer induction. Further support for a cofactor role for sunlight comes from the finding of papillomavirus in ocular tumors of cattle exposed to the sun [37], in skin cancers arising in sheep in areas not protected by wool or pigment [38], and in solar keratoses in humans [39]. Still another cofactor role for sunlight exposure might be further immunosuppression by UV light [40,41].

To date, no effective treatment for patients suffering with EV has been available, except for the surgical removal of skin cancers. Retinoids (synthetic vitamin A) have been found recently to be successful in treating disorders of keratinization [42] and to modify the terminal differentiation of keratinocytes [43]. Since the HPVs replicate exclusively in keratinizing cells and produce changes in keratinization [1,11,15,16,24], we attempted treatment of four EV patients with etretinate (Tigason), an aromatic synthetic retinoid. Our results showed the drug to be effective in ameliorating but not curing the benign lesions, as reported also by others [44], and in one patient, the retinoids proved effective in reducing the viral content [45]. Two Bowenoid in situ cancers reduced in size [45]. This may be related to the observation that Shope papillomavirus-induced warts in rabbits [46] and chemically induced skin tumors in mice [47] could be substantially reduced in size by retinoids. The beneficial effect of retinoids in EV might result from a modification of terminal differentiation [43], a direct antiviral action [48], or the enhancement of killer T cells [49]. It should be determined whether or not sustained therapy with Tigason might reduce the incidence of new cancers.

Since it has been reported that a child with extensive nasopharyngeal carcinoma, associated with EBV, had responded successfully to interferon [50] and that interferon had been helpful in treating children suffering with massive laryngeal papillomas [51], we decided to try this antiviral-antitumor agent [52] in EV patients with cancers. We found that small Bowenoid cancers in situ responded well to intralesional interferon injections, but a large invasive squamous cell carcinoma responded only partially and transiently [53].

In conclusion, the interaction between papillomavirus infection, genetic and immunologic factors, and sunlight in EV patients presents a model for determining the role of these factors in provoking skin cancer in humans.

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